claim in the new claims (claim 237) by rewriting the claim as an independent claim.

For the foregoing reasons, Applicants submit that the objections under 37 CFR 1.7 5(c) have been overcome and respectfully request reconsideration and withdrawal of the objections.

II. The Rejection of Claims 174, 184-188, and 193 under 35 U.S.C. § 112, First Paragraph

Claims 174, 184-188, and 193 stand rejected under 35 U.S.C. § 112, first paragraph, "as containing subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor(s), at the time the application was filed, had possession of the claimed invention. The Office Action states:

> The specification describes a single polypeptide sequence consisting of SEQ ID NO:2, which is shown to have the activities as recited in claim 170 elements i-iv/claim 184 elements a)-d). However, the claims are directed to peptides encoded by nucleic acid sequences which hybridizes with SEQ ID NO:1 or it's complementary strand, and to fragments which exhibit amino peptidase activity. In addition, in contrast to claim 170, claims 174, 184-187 and 193 lack any structural recitations in the claims (including fragments with aminopeptidase activity) and thus encompass any structure which achieves the functional characteristics. Thus, the claims encompass all structural molecules which share the delimited functional constraints. Yet, the instant disclosure of a single polypeptide, that of SEQ ID NO:2 with the instantly disclosed specific activities, does not adequately describe the claimed genus drawn to a substantial variety of subgenera.

This rejection is respectfully traversed.

Applicants assert the same arguments on record in the amendment of January 23, 2002 that limiting the claims to SEQ ID NOs:1 and 2 would not adequately protect the inventors. Based on the teachings of the present application, one skilled in the art could find another aminopeptidase having the properties of the aminopeptidases of the present invention and thereby attempt to circumvent the literal scope of Applicants' patent rights. Thus, a competitor seeking to avoid infringing the claims would merely have to follow the disclosure in the subsequently-issued patent to find a substitute.

The Office Action states "in claims 174, 184-187 and 193 there are no distinguishing structural features noted for the subgenera of functional variants or for functional fragments. The specification describes only a singular species which falls within the subgenus but fails to describe the structural features commonly possessed by its members or an adequate number of species which distinguish the subgenus members from others, including fragments and sequences which provide for the recited activity. Thus, as in *Regents of the University of California* v *Eli Lilly & Co,* 119 F.3d 1559, 1569, 43 USPQ.2d 1398, 1406 (Fed. Cir. 1997), the naming of the type of material generally known to exist, in absence of knowledge as to what that material consists of, is not a description of that material."

Applicants disagree with this assertion. *The Regents of the University of California v. Eli Lilly & Co.* court stated: "An adequate written description of DNA that is subject of patent requires precise definition, such as by structure, formula, chemical name, **or** physical properties." *The Regents of the University of California,* 119 F.3d 1559, 1566. The court did not state "by structure **and** physical properties." Claims 174, 184, and 193 ultimately depend on claim 170. Claim 170's preamble recites that the isolated secreted polypeptide having aminopeptidase activity has physicochemical properties of (i) a pH optimum in the range of from about pH 7.27 to about pH 10.95 determined at ambient temperature in the presence of Ala-para-nitroanilide; (ii) a temperature stability of 90% or more, relative to initial activity, at pH 7.5 determined after incubation for 20 minutes at 60°C in the absence of substrate; (iii) a temperature stability of 64% or more, relative to initial activity, at pH 7.5 determined after incubation for 20 minutes at 70°C in the absence of substrate; and (iv) an ability to hydrolyze a substrate containing Ala, Arg, Asn, Asp, Cys, Gln, Glu, Gly, His, Ile, Leu, Lys, Phe, Pro, Ser, Thr, Trp, Tyr, or Val at its N-terminus." Applicants contend that the above-noted properties recited in the claims meet this requirement. However, to further prosecution, Applicants have deleted claims 184-187. To overcome the rejection of claims 174 and 193, which have been cancelled, new claims 211 and 227 (corresponding to claims 174 and 193, respectively) recite "a fragment of contiguous amino acids of amino acids 16 to 496 of SEQ ID NO:2 wherein the fragment has aminopeptidase activity."

The Office Action also states: "In addition, in contrast to claim 170, claims 174, 184-187 and 193 lack any structural recitations in the claims (including fragments with aminopeptidase activity) and thus encompass any structure which achieves the functional characteristics." The term "fragment" in claims 174 and 193 is defined on page 26, lines 21-31, of the specification as "a polypeptide having one or more amino acids deleted from the amino and/or carboxy terminus of this amino acid sequence. ... Preferably, a fragment contains at least 330 amino acid residues, more preferably at least 380 amino acid residues, and most preferably at least 430 amino acid residues." One skilled in the art could modify an aminopeptidase of the present invention, such as amino acids 16 to 496 of SEQ ID NO:2 by removing one or more amino acids form the N- or C-terminus regions while maintaining the above-noted properties and thereby circumvent the literal scope of Applicants' patent rights. To overcome the rejection, as described above, claims 174 and 193 have been cancelled but new claims 211 and 227 recite "a fragment of contiguous amino acids of amino acids 16 to 496 of SEQ ID NO:2 wherein the fragment has aminopeptidase activity."

The Office Action also states: "In addition, the specification fails to delineate hybridizing sequences other than that of SEQ ID NO: 1 which meet the functional constraints. It is again noted that sequences hybridizing to SEQ ID NO: 1 reveal non-coding strands unrelated to the polypeptide

claimed, and thus the claims lack written description of a representative number of species of the hybridizing or fragment subgenus." Applicants respectfully disagree with this assertion. Preliminarily, Applicants detail on page 5, line 18, to page 6, line 17, of the specification, instructions for performing standard Southern hybridization under medium and high stringency conditions to identify other nucleic acids encoding aminopeptidases having the properties as claimed herein using nucleotides 46 to 1488 of SEQ ID NO:1, or its full complementary strand, as a probe. One of ordinary skill in the art would recognize that the use of such a probe under medium, medium-high, or high stringency conditions allows the identification of genes encoding other aminopeptidases that are very closely related or essentially identical to the gene contained in SEQ ID NO:1. For example, Applicants have provided details in Example 7 for probing a library of an *Aspergillus* strain where eleven colonies produced strong hybridization signals with the probe. Applicant disagrees with the Office Action's statement that "sequences hybridizing to SEQ ID NO: 1 reveal non-coding strands unrelated to the polypeptide claimed." The claims recite in the preamble "[a]n isolated secreted polypeptide having aminopeptidase activity with physicochemical properties of (i) a pH optimum in the range of from about pH 7.27 to about pH 10.95 determined at ambient temperature in the presence of Ala-para-nitroanilide; (ii) a temperature stability of 90% or more, relative to initial activity, at pH 7.5 determined after incubation for 20 minutes at 60°C in the absence of substrate; (iii) a temperature stability of 64% or more, relative to initial activity, at pH 7.5 determined after incubation for 20 minutes at 70°C in the absence of substrate; and (iv) an ability to hydrolyze a substrate containing Ala, Arg, Asn, Asp, Cys, Gln, Glu, Gly, His, Ile, Leu, Lys, Phe, Pro, Ser, Thr, Trp, Tyr, or Val at its N-terminus" and, thus, any non-coding strands would fall outside the scope of the claims. However, under the stringency conditions used by Applicants in conjunction with nucleotides 46 to 1488 of SEQ ID NO:1, or its full complementary strand, as a probe, only nucleic acid sequences encoding polypeptides having aminopeptidase activity with the above-noted properties will be identified (see section V below). Moreover, small oligonucleotides such as the 30mer cited by the Office Action (page 8) would not hybridize with nucleotides 46 to 1488 of SEQ ID NO:1, or its full complementary strand, under the conditions claimed herein.

The Office Action further states: "In claim 188, there is no description of a suitable strain other than *Aspergillus oryzae* or deposited strain pEJG18, *E. coli* NRRL B-21677." Applicants have cancelled claim 188 but new claim 222 recites: "A method for producing the secreted polypeptide of claim 207 comprising (a) cultivating a microbial strain, which in its wild-type form produces the polypeptide, in a medium under conditions suitable for production of the polypeptide; and (b) recovering the polypeptide from the medium." Applicants disclose on page 8, line 21, to page 10, line 29, a list of microorganisms, which, in their wild-type form, may produce the polypeptides of the present invention.

These microorganisms include bacteria and fungi. Applicant asserts that any microorganism in its wild-type form that comprises, for example, nucleotides 46 to 1488 of SEQ ID NO:1 and its full complementary strand, is well within the skill in the art to be cultivated in a medium suitable for expression of the polypeptide and recovered therefrom.

Applicants submit that the information disclosed in the specification combined with the knowledge of the art provides sufficient guidance to inform the skilled artisan that Applicants were in possession of the claimed aminopeptidases at the time the application was filed.

For the foregoing reasons, Applicants submit that the rejections under 35 U.S.C. § 112, first paragraph, have been overcome and respectfully request reconsideration and withdrawal of the rejections.

III. The Rejection of Claims 174, 184-188, and 193 under 35 U.S.C. § 112, First Paragraph

Claims 174, 184-188, and 193 stand rejected under 35 U.S.C. § 112, first paragraph, because "the specification, while being enabling for the aminopeptidase of residues 16-496 of SEQ ID NO:2, does not reasonably provide enablement for the generic recitation of any polypeptide providing the functional characteristics noted in a)-d)." Specifically, the Office Action states:

> [I]n view of the lack of guidance, lack of examples, and lack of predictability associated with regard to producing and using the myriad of derivatives and fragments encompassed by the claims, one skilled in the art would be forced into further undue experimentation in order to determine those peptides which correlate to the recited functional characteristics of the claimed genus and a method of producing and recovering such in order to practice the claimed invention.

This rejection is respectfully traversed.

Preliminarily, claims 184-188 have been cancelled as noted in Section II rendering their rejection moot. The Examiner suggests that the specification is insufficient to enable one skilled in the art to practice the invention as broadly claimed without undue experimentation. Applicants respectfully disagree. Applicants submit that undue experimentation would not be required to practice the invention because Applicants' disclosure provides considerable direction and guidance on how to practice their invention and presents working examples. There was a high level of skill in the art at the time when the application was filed, and Applicants' enabling disclosure in combination with that skill in the art provides sufficient knowledge needed to practice the invention.

Applicants have provided detailed methods for isolating the claimed aminopeptidase and determining whether they fall within the scope of protection sought by Applicants using Applicants'

enabling methods for preparing and probing DNA libraries (see Example 4-7); for isolating nucleic acids encoding the aminopeptidase (see Example 7); for determining cross-hybridization of the nucleic acids encoding the aminopeptidase using nucleotides 46 to 1488 of SEQ ID NO:1, or their complementary nucleotides (se page 5, line 18, to page 6, line 18); for comparing the percent identity of the deduced amino acid sequence of the aminopeptidase to amino acids 16 to 496 of SEQ ID NO:2 using the Clustal method according to Higgins, 1989, *CABIOS* 5: 151-153 (see page 4, line 9, to page 5, line 17); for expressing the nucleic acid sequence encoding an aminopeptidase in a host cell (see Examples 9-10 and 12-14); for purifying the aminopeptidase (see Example 11); and for characterizing the properties of the aminopeptidase (see Example 16). Applicants assert that it is well within the skill of the art to practice the invention using the Applicants' enabling disclosure without undue experimentation. The methods for isolating the claimed aminopeptidases and determining their properties are described in the specification in enabling detail for practicing the claimed invention. On the basis of Applicants' disclosure, one skilled in the art would know how to identify and isolate such aminopeptidases. Applicants, therefore, submit that the information disclosed in the specification enables one skilled in the art to isolate the claimed aminopeptidases.

The Office Action suggests that claims 174, 184-187 and 193, drawn to any polypeptides or polypeptide fragments which provides for the functional characteristics of elements a)-d) or aminopeptidase activity, are akin to a single means claim and subject to an undue breadth rejection under 35 U.S.C. § 112, first paragraph, because the claim depends on a recited property and covers every conceivable structure (means) for achieving the stated property (result) while the specification discloses only SEQ ID NO:2. Applicants respectfully disagree. Claims 174 and 193 have been redrafted as new claims 211 and 227. Claim 211 recites "The polypeptide of claim 207, comprising the amino acid sequence of amino acids 16 to 496 of SEQ ID NO:2 or a fragment of contiguous amino acids of amino acids 16 to 496 of SEQ ID NO:2 wherein the fragment has aminopeptidase activity." New claim 227 uses the same language. The fragment aspect of the claims relates directly to amino acids 16 to 496 of SEQ ID NO:2. Thus, the claims depend both on amino acids 16 to 496 of SEQ ID NO:2 and the recited properties and do not cover every conceivable structure (means) for achieving the stated property. Based on Applicants' disclosure, it is well within the skill in the art to determine whether a fragment of amino acids 16 to 496 of SEQ ID NO:2 falls within the scope of the claims without any undue experimentation. Limiting the claims to amino acids 16 to 496 of SEQ ID NO:2, one skilled in the art could modify amino acids 16 to 496 of SEQ ID NO:2 by removing one or more amino acids from the N- or C-terminus regions while maintaining the above-noted properties and thereby circumvent the literal scope of Applicants' patent rights.

The Office Action also suggests the unpredictable nature of hybridization in that there exists multiple variability in hybridizing conditions and the ability of divergent sequences to hybridize based on the length of the sequences, mismatch base pairing, temperature and unique binding characteristics of the sequences and the artisan could not a priori determine those sequences capable of hybridization at any given set of conditions which also shared the functional constraints of the peptides claimed. Applicants respectfully disagree with this assertion. Applicants have defined the conditions under which hybridization must be carried out to determine whether a nucleic acid sequence falls within the scope of the claims. Applicants have also defined the properties that the encoded polypeptide must have to fall within the scope of the claims. Moreover, using nucleotides 46 to 1488 of SEQ ID NO:1, or its full complementary strand, as the probe under the specified conditions as claimed herein, one of ordinary skill in the art would recognize that the use of such a probe under medium or high stringency conditions allows the identification of genes encoding other aminopeptidases that are very closely related or essentially identical to the gene contained in SEQ ID NO:1. Applicants' position is further buttressed by the argument Applicants provide in Section V. See Section V.

The Office Action further suggests concerning claim 188 that the artisan would be required to perform further undue experimentation to first determine those peptides which correlate to the specifications of the claims and then determine an adequate means of producing the peptide, including either its sequence structure or the microorganism capable of its production. Applicants disagree with the Office Action's contention. New claim 222, which corresponds to claim 188, now recites: "A method for producing the secreted polypeptide of claim 170 comprising (a) cultivating a microbial strain, which in its wild-type form produces the polypeptide, in a medium under conditions suitable for production of the polypeptide; and (b) recovering the polypeptide from the medium." In the first place, the microbial strain in its wild-type form produces the polypeptide. There is no need, therefore, to determine an adequate means of producing the peptide. For the secreted polypeptide to be isolated, it was necessarily produced by the microbial strain in its wild-type form when cultivated. Moreover, once isolated, a determination of the properties of the polypeptide and its sequence is well within the skill of the art using Applicants' specification as a guide. These determinations do not represent undue experimentation, but are rather routine determinations generally performed.

Applicants, therefore, assert that the specification is sufficiently enabling to practice the claimed invention. For the foregoing reasons, Applicants submit that the rejections under 35 U.S.C. § 112, first paragraph, have been overcome and respectfully request reconsideration and withdrawal of the rejections.

IV. The Rejection of Claim 188 under 35 U.S.C. § 112, Second Paragraph

Claim 188 stands rejected under 35 U.S.C. § 112, second paragraph, as being indefinite "for omitting essential elements, such omission amounting to a gap between the elements." The Office Action states that the omitted elements are "the strain or source (of micro-organism) required to produce a supernatant comprising the polypeptide of claim 170" and "those steps comprising 'cultivating' suitable to produce a supernatant comprising the polypeptide from any particular strain or micro-organism."

Applicants have amended claim 188 to recite: "A method for producing the polypeptide of claim 170 comprising (a) cultivating a microbial strain, which in its wild-type form produces the polypeptide, in a medium under conditions suitable for production of the polypeptide; and (b) recovering the polypeptide from the medium." As noted above in Section II., Applicants disclose on page 8, line 21, to page 10, line 29, a list of microorganisms, which, in their wild-type form, may produce the polypeptides of the present invention. These microorganisms include bacteria and fungi. Applicant asserts that any microorganism in its wild-type form that comprises, for example, nucleotides 46 to 1488 of SEQ ID NO:1 and its full complementary strand, is well within the skill in the art to be cultivated in a medium suitable for expression of the polypeptide and recovered therefrom.

For the foregoing reasons, Applicants submit that the new claims overcome the rejections under 35 U.S.C. § 112. Applicants respectfully request reconsideration and withdrawal of the rejection.

V. The Rejection of Claims 170, 74, 77,180,184-185,188,189, 193, 196 and 199 under 35 U.S.C. § 102

Claims 170, 74, 77,180,184-185,188,189, 193, 196 and 199 stand rejected under 35 U.S.C. § 102(b) as being anticipated by Nishizawa *et al.* (J. Biol. Chem. 269:13651-13655, 1994). The Office Action states:

> Nishizawa teach a *S. cerevisiae* aminopeptidase which hybridizes with SEQ ID NO: 1 as the Nishizawa sequence encodes residues 255-264 of SEQ ID NO:2. This 30 mer has a Tm=87 degrees C based on the formula Tm= 4(G+C)+2(A+T) and as it meets such characteristics is expected to have aminopeptidase activity, absent factual evidence to the contrary. As the Tm is above the medium and high temperature stringency, the sequence inherently hybridizes under medium and high stringency conditions of 42 degrees C, absent factual evidence to the contrary. Thus, the sequence anticipates the claimed invention, see in particular residues 1180-1209 of the Nishizawa nucleic acid sequence.

This rejection is respectfully traversed.

Under the standard required for anticipation under 35 U.S.C. § 102, the cited prior art

reference is required to disclose every element of the claimed invention. *Lewmar Marine Inc. v. Barient Inc.*, 3 USPQ2d 1766 (Fed. Cir. 1987).

Nishizawa *et al.* disclose a *Saccharomyces cerevisiae* aminopeptidase Y, a vacuolar enzyme consisting of 537 amino acids. However, Nishizawa *et al.* do not disclose every element of the invention claimed herein.

First, the claimed aminopeptidases are not vacuolar enzymes. The claimed aminopeptidases are secreted enzymes unlike the aminopeptidase Y of Nishizawa *et al.*, which is not secreted.

Second, the Office Action contends that a 30 mer of the Nishizawa sequence, *i.e.*, nucleotides 1180-1209, which encodes residues 255-264 of SEQ ID NO:2, has a Tm=87°C based on the formula Tm= 4(G+C) + 2(A+T) and as it meets such characteristics is expected to have aminopeptidase activity. Applicant respectively disagrees with this contention because the 30 mer of the Nishizawa sequence does not comport with what Applicant has claimed. Claim 1(b) recites "a polypeptide which is encoded by a nucleic acid sequence which hybridizes under medium stringency conditions with (i) the nucleic acid sequence of nucleotides 46 to 1488 of SEQ ID NO:1, or (ii) its full complementary strand, wherein medium stringency conditions are defined as prehybridization and hybridization at 42°C in 5X SSPE, 0.3% SDS, 200 µg/ml sheared and denatured salmon sperm DNA, and 35% formamide." The claim means that a polypeptide having aminopeptidase activity is encoded by a nucleic acid sequence which hybridizes under medium stringency conditions with nucleotides 46 to 1488 of SEQ ID NO:1. The Office Action merely mentions a 30 mer which encodes 10 amino acids. A peptide of ten amino acids is far too small to have aminopeptidase activity. Moreover, the 30 mer cited by the Office Action would not hybridize with nucleotides 46 to 1488 of SEQ ID NO:1 under the conditions claimed herein (see below).

Third, the Office Action uses the formula Tm = 4(G+C)+2(A+T), which is not appropriate in the present case because the formula is used for short oligonucleotides and does not take into account the presence of salt and formamide during hybridization. A more appropriate formula for determining the effective Tm is as follows:

Effective Tm = 81.5 + 16.6(log M[Na^+]) + 0.41(%G+C) – 0.72(%formamide)

(See www.ndsu.nodak.edu/instruct/mcclean/plsc731/dna/dna6.htm).

The G+C content of SEQ ID NO:1 or nucleotides 46 to 1488 of SEQ ID NO:1 is 58%. For medium stringency, the % formamide is 35% and the Na^+ concentration for 5X SSPE is 0.75 M. Applying this formula to these values, the Effective Tm is 78°C. Another relevant relationship is that a 1% mismatch of two DNAs lowers the Tm 1.4°C. To determine the degree of homology required for the two DNAs to hybridize under medium stringency conditions at 42°C, the following formula is used:

% Homology = 100 – [(Effective Tm – Hybridization Temperature)/1.4]

(See www.ndsu.nodak.edu/instruct/mcclean/plsc731/dna/dna6.htm)

Applying this formula to the values, the degree of homology required for the two DNAs to hybridize under medium stringency conditions at 42°C is 100 – [(78 – 42)/1.4] = 74.3%. Since there is no obvious homology between SEQ ID NO:1 and Nishizawa *et al.'s* DNA sequence encoding aminopeptidase Y, the two sequences will not hybridize under medium stringency conditions.

For the foregoing reasons, Applicants submit that this rejection under 35 U.S.C. § 102 has been overcome. Applicants respectfully request reconsideration and withdrawal of the rejection.

VI. Conclusion

In view of the above, it is respectfully submitted that all claims are in condition for allowance. Early action to that end is respectfully requested. The Examiner is hereby invited to contact the undersigned by telephone if there are any questions concerning this amendment or application.

Respectfully submitted,

Date: November 26, 2002

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